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JUL 2 7 2001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Jack **LILIEN** *et al.*

Serial No. 09/836,865

Filed: April 18, 2001

For: SYSTEM TO DETECT PROTEIN-
PROTEIN INTERACTIONS

Art Unit: 1642

Examiner: unknown

Atty. Docket No. 38368-171364

Customer No.

26694
PATENT TRADEMARK OFFICE

PRELIMINARY AMENDMENT

Honorable Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination on the merits, please enter the following amendments to the claims. These amendments are being made to reduce the filing fees. It is understood that it is proper to adjust the filing fee, if not paid at the time of filing, if the number of claims is reduced by the time the filing fee is paid in response to a "Notice to File Missing Parts..."

IN THE CLAIMS:

[A full set of claims appears below, including retained (unamended) claims]

Please amend the claims as follows:

1. A screening method for identifying, in a library of potential binding domains (PBDs) from a biological source, a polypeptide binding domain or domains that bind to a target epitope or family of target epitopes, comprising:

(a) providing a cDNA library from said source that encodes said library of PBDs as a T7 phage display library wherein the PBDs are displayed on the outer surface of said T7 phages as fusion proteins with an outer surface protein (OSP) of said T7 phages;

(b) contacting said phage display library with a bindable array of target epitopes or families of epitopes under conditions where any of said PBDs binds to said target epitopes;

(c) removing unbound T7 phages from said array of target epitopes, so that phages remaining bound are a first sublibrary enriched for PBD-displaying phages;

(d) eluting bound T7 phage from said array of target epitopes ; and

(e) determining the DNA sequence encoding the PBDs from said first sublibrary of eluted T7 phage, thereby identifying the PBDs displayed on said eluted phage by their predicted amino acid sequence.

2. The method of claim 1 wherein at least one of

(i) the PBDs of step (a), or

(ii) the target epitope or family of step (b)

are predetermined.

3. The method of claim 2, wherein said target epitope or family of epitopes are predetermined.

4. The method of claim 3 comprising, after said eluting step (d) and before said determining step (e), the step of:

(f) subjecting said eluted phage to at least one additional round of contacting and removing of steps (b) and (c) to further enrich phage displaying said PBDs that bind to set predetermined target epitope or epitopes, thereby obtaining a second sublibrary and subsequent sublibraries.

5.   The method of claim 4, wherein step (f) is repeated more than once prior to said determining step (e), after each repeat obtaining a new subsequent sublibrary.

6. *(amended)*   The method of claim 1, wherein said outer surface protein capsid protein encoded by gene 10A or 10B of phage T7.

7.   The method of claim 6, wherein said outer surface protein capsid protein encoded by gene 10B of phage T7.

8.   The method of claim 7, wherein in said display library, said PBDs are expressed in a copy number of about 5-10 PBDs per phage particle.

9.   The method of claim 7, wherein, in said phage display library, said PBDs are expressed in high copy number of 415 PBDs per page particle.

10.   The method of claim 7, wherein in said phage display library, said PBDs are expressed in an intermediate copy number of about 100 to about 150 PBDs per page particle.

11. *(amended)*   The method of claim 1, wherein said determining step (e) is performed by plating said eluted phage on a lawn of *E. coli*, permitting them to multiply and form plaques, and sequencing the DNA of the phages of any given plaque to obtain the sequence of the cDNA insert that encodes said PBD.

12. *(amended)*   The method of claim 1, wherein said target epitopes are peptide epitopes and said family comprises peptides or polypeptides corresponding to (i) a protein fragment, (ii) a protein domain or (iii) a complete protein.

13.   The method of claim 12, wherein said family of target peptide epitopes comprises a progressive series of overlapping peptides of about 10 to 15 amino acids, each of which peptides lacks *n* amino-terminal amino acid residues of its predecessor peptide in the series and has at least *n* additional amino acids added to its carboxy-terminus, wherein *n* is an integer between 1 and 5, , and wherein said series of overlapping peptides corresponds to (i) a region of said protein of up to about 100 amino acids, or (ii) said complete protein.

14.   The method of claim 12, wherein said target peptides are synthesized in parallel on polyethylene pins mounted on blocks which are compatible with standard microplate arrays of 96 wells or multiples thereof.

15.   The method of claim 13, wherein said target peptides are synthesized in parallel on polyethylene pins mounted on blocks which are compatible with standard microplate arrays of 96 wells or multiples thereof.

16.   The method of claim 14, wherein the target peptides are covalently attached to the pins so that said, after said eluting of said bound phages, the blocks are reused for one or more additional screening assays.

17.   The method of claim 15, wherein the target peptides are covalently attached to the pins so that said, after said eluting of said bound phages, the blocks are reused for one or more additional screening assays.

18.   The method of claim 17, wherein the target peptides are in a cleavable form, allowing recovery of said peptides.

19. *(amended)*   The method of claim 1, wherein said cDNA library is produced from mRNA molecules of said biological source by random priming wherein each cDNA molecule reverse transcribed from said mRNA molecules is between about 50 and about 5000 bp in length, the cDNA molecules are gel purified and directionally cloned into said T7 phage DNA resulting in fused DNA, and said fused DNA is packaged into phage *in vitro.*

20.   The method of claim 19, wherein the cDNA molecule is between about 50 and about 1000 bp in length.

21.   The method of claim 20, wherein the cDNA molecule is between about 50 and 500 bp in length.

22.   The method of claim 21, wherein the cDNA molecule is between about 100 and 200 bp in length.

23. *(amended)*   A method to determine the representation of expressed sequences in a PBD display sublibrary, when said PBDs are from a known protein and specific antibodies for epitopes of the known protein are available,

(i)   providing a collection of antibodies specific for the epitopes of the known protein which antibodies are immobilized to a solid support;

(ii)   carrying out the method of claim 4 up to an eluting step wherein the first sublibrary, the second sublibrary or a subsequent sublibrary is obtained;

(iii)   contacting the sublibrary obtained in step (ii) with the antibodies of step (i) and permitting the antibodies to bind to the epitopes of the displayed PBDs

(iv)   evaluating the results of the binding, thereby determining the representation of the expressed sequences in said sublibrary.

24.   The method of claim 23, wherein the solid support is magnetic beads.

25.   The method of claim 23, comprising, in addition to the antibody binding steps, the step of obtaining multiple separate phage clones from the sublibrary, separately isolating the DNA therefrom, and sequencing the cDNA insert of each clone that encodes the PBD of that clone.

26. *(amended)*   The method of claim 1 wherein the biological source is selected from the group consisting of developing chick neural retina, cultured neonatal rat Schwann cells, and myelinating sciatic nerves of 15-25 day old rat.

27.   The method of claim 26, wherein the biological source is the Schwann cells or the sciatic nerves, and the target epitopes are peptides of a peripheral myelin protein selected from the group of proteins consisting of PMP22, P0, connexin 32 and EGR2.

28.   The method of claim 26, wherein the target epitopes are peptides from the cytoplasmic domain of peripheral myelin protein P0.

29. *(amended)*   The method of claim 1, wherein

(a)   the phage display library displays PBDs of a protein selected from the group consisting of β-catenin, PTP1B, p120ctn and Shc; and

(b)   the target epitopes are peptides of N-cadherin.

30. *(amended)* The method of claim 1, wherein

(a) the phage display library displays PBDs of synaptotagmin SytI and the target epitopes are peptides of synaptotagmin Syt IV; or

(b) the phage display library displays PBDs of SytIV and the target epitopes are peptides of Syt I.

31. *(amended)* The method of claim 1, wherein

(a) the phage display library displays PBDs of SytI or Syt IV and the target epitopes are peptides of syntaxin; or

(b) the phage display library displays PBDs of syntaxin and the target epitopes are peptides of Syt I or Syt IV.

32. A method of identifying peptides participating in protein-protein interactions by screening a first peptide display library for members that interact with a second peptide display library, the method comprising

(a) providing a first cDNA library from a biological source that encodes PBDs as a first T7 phage display library wherein the PBDs are displayed on the outer surface of said T7 phages as fusion proteins with an outer surface protein of said T7 phages, which first display library is immobilized to a solid support and said PBDs are available for binding to a peptide for which they have binding specificity;

(b) providing the second library which is a combinatorial library of peptides displayed on genetic display packages other than T7 that are available for binding to the immobilized members of said first library;

(c) contacting the members of said immobilized T7 first library with members of said second library;

(d) removing unbound particles of both of said libraries so that second library particles remaining bound are enriched for those displaying peptides that bind to the PBDs displayed on the T7 phages,

(e) eluting the bound particles

(f) selectively growing the T7 phages and said genetic display packages under conditions wherein either the T7 phages or the genetic display packages have a

growth advantage to obtain enriched populations of the T7 phages expressing said first library and the genetic display packages expressing said second library;

(g)   separately amplifying the DNA of the second library particles and the immobilized first library phages to which the second library particles had been bound, and sequencing amplified DNA libraries, thereby determining the predicted amino acid sequences of

(i)   the PBDs normally expressed in the biological source that participate in said protein-protein interactions with said second library peptides, and

(ii)   the peptides that are part of, or that mimic, endogenous proteins that normally interact with said first library PBDs

thereby identifying the peptides participating in the protein-protein interactions

33.   The method of claim 32, wherein immobilization is by an antibody specific for an outer surface structure of said T7 phage

34.   The method of claim 33, wherein said outer surface structure is a tail fiber.

35.   The method of claim 32, wherein said genetic display package is a phage.

36.   The method of claim 35, wherein the phage is M13.

37.   The method of claim 36, wherein the second library is an M13 random combinatorial peptide library.

38.   The method of claim 37, wherein members of said second library have from about 4 to about 30 amino acids with a complexity of expressed peptides of between about 10^7 and about 10^{15}.
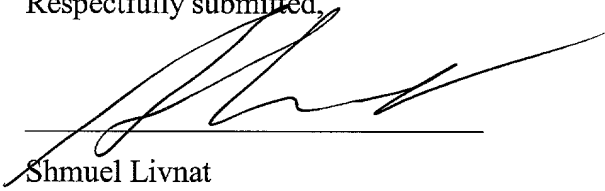
REMARKS

As noted above, the amendments of the claims set forth above were made strictly to remove or decrease certain multiple dependencies, thereby reducing the total number of claims the extra claim fees.

It is respectfully submitted that the present claims are in condition for examination on the merits. However, if any questions remain, the Examiner or other PTO official is encouraged to call the undersigned at (202) 216-8584 to expedite this application.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorizes the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 22-0261.** However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Respectfully submitted,

Date: July 27, 2001

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APPENDIX
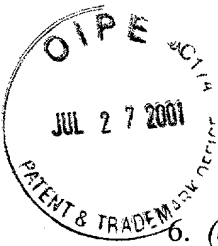
AMENDED CLAIMS ONLY
(with markings to show changes)

6. *(amended)*   The method of [any of] claim[s] 1[-5] wherein said outer surface protein capsid protein encoded by gene 10A or 10B of phage T7.

11. *(amended)*   The method of [any of] claim[s] 1[-5], wherein said determining step (e) is performed by plating said eluted phage on a lawn of *E. coli*, permitting them to multiply and form plaques, and sequencing the DNA of the phages of any given plaque to obtain the sequence of the cDNA insert that encodes said PBD.

12. *(amended)*   The method of [any of] claim[s] 1[-5], wherein said target epitopes are peptide epitopes and said family comprises peptides or polypeptides corresponding to (i) a protein fragment, (ii) a protein domain or (iii) a complete protein.

19. *(amended)*   The method of [any of] claim[s] 1[-5], wherein said cDNA library is produced from mRNA molecules of said biological source by random priming wherein each cDNA molecule reverse transcribed from said mRNA molecules is between about 50 and about 5000 bp in length, the cDNA molecules are gel purified and directionally cloned into said T7 phage DNA resulting in fused DNA, and said fused DNA is packaged into phage *in vitro*.

23. *(amended)*   A method to determine the representation of expressed sequences in a PBD display sublibrary, when said PBDs are from a known protein and specific antibodies for epitopes of the known protein are available,

     (i)   providing a collection of antibodies specific for the epitopes of the known protein which antibodies are immobilized to a solid support;

     (ii)   carrying out the method of claim 4 [5 or 6] up to an eluting step wherein the first sublibrary, the second sublibrary or a subsequent sublibrary is obtained;

     (iii)   contacting the sublibrary obtained in step (ii) with the antibodies of step (i) and permitting the antibodies to bind to the epitopes of the displayed PBDs

     (iv)   evaluating the results of the binding, thereby determining the representation of the expressed sequences in said sublibrary.

APPENDIX

26. *(amended)*   The method of [any of] claim[s] 1[-5] wherein the biological source is selected from the group consisting of developing chick neural retina, cultured neonatal rat Schwann cells, and myelinating sciatic nerves of 15-25 day old rat.

29. *(amended)*   The method of [any of] claim[s] 1[-5], wherein

(a)   the phage display library displays PBDs of a protein selected from the group consisting of β-catenin, PTP1B, p120ctn and Shc; and

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30. *(amended)*   The method of [any of] claim[s] 1[-5], wherein

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31. *(amended)*   The method of [any of] claim[s] 1[-5], wherein

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